

# Herd-level spatial cluster analysis of bovine cysticercosis in the state of Paraíba, northeastern Brazil

Análise de aglomerados espaciais no nível do rebanho de cisticercose bovina no Estado da Paraíba, Nordeste do Brasil

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## Abstract

The aim of this survey was to identify spatial clustering of bovine cysticercosis-positive herds in the state of Paraíba. The state was divided into three sampling groups: sampling stratum 1 (Sertão mesoregion), sampling stratum 2 (Borborema mesoregion) and sampling stratum 3 (Zona da Mata and Agreste mesoregions), and 2382 cows aging  $\geq 24$  months from 474 farms were sampled. Serological diagnoses of bovine cysticercosis were initially done by means of indirect ELISA, and positive serum samples were confirmed by a immunoblot test. Herds were deemed positive for cysticercosis if they presented at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females. The spatial clustering was assessed using the Cuzick-Edwards  $k$ -nearest neighbor method and spatial scan statistics. A significant clustering of positive herds was detected in the southern part of the Borborema mesoregion. Given that serological tests for bovine cysticercosis are not widely available, and also that replacement and maintenance of herds through animal purchases is common in the region, it can be concluded that prevention measures should be applied at herd level.

**Keywords:** Cattle, epidemiology, cluster analysis, bovine cysticercosis.

## Resumo

O objetivo deste estudo foi identificar agrupamentos espaciais de rebanhos positivos para cisticercose bovina no Estado da Paraíba. O Estado foi dividido em três grupos amostrais: estrato amostral 1 (mesorregião do Sertão), estrato amostral 2 (mesorregião da Borborema), e estrato amostral 3 (mesorregiões da Zona da Mata e Agreste), e 2.382 vacas com idade  $\geq 24$  meses foram amostradas a partir de 474 propriedades. O diagnóstico sorológico da cisticercose bovina foi, inicialmente, realizado pelo ELISA indireto, e as amostras de soro positivas foram confirmadas por imunoblot. Um rebanho foi considerado positivo para cisticercose, se apresentasse pelo menos um animal positivo em rebanhos de até 29 fêmeas; e dois animais positivos em rebanhos com mais de 29 fêmeas. Os agrupamentos espaciais foram avaliados com o uso da metodologia  $k$ -vizinhos mais próximos de Cuzick-Edwards e estatística espacial de varredura. Um agrupamento significativo de rebanhos positivos foi detectado na parte sul da mesorregião da Borborema. Tendo em vista que os testes sorológicos para diagnóstico de cisticercose bovina não são amplamente disponíveis, bem como é comum na região a reposição e manutenção dos rebanhos por compra de animais, conclui-se que medidas de prevenção devem ser aplicadas em nível de rebanho.

**Palavras-chave:** Gado, epidemiologia, análise de cluster, cisticercose bovina.

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## Introduction

Bovine cysticercosis is a tropical zoonotic disease caused by the larval stage of *Taenia saginata* in cattle. Its adult phase causes taeniasis in humans (CALVO-ARTAVIA et al., 2013). Cattle become infected by consuming contaminated water or by feeding on pasture containing viable eggs of the parasite or through any other manner that leads to intake of these eggs. Despite the limitations, postmortem inspection has been used as a control measure and it indicates the degree of bovine cysticercosis infection. Therefore, visual inspection of beef carcasses during slaughter is very important for reducing the risk for consumers (COSTA et al., 2012), since this zoonosis has great public health importance in developing countries and causes economic losses to the beef supply chain (LARANJO-GONZÁLEZ et al., 2016).

Data on bovine cysticercosis in Brazil is available only from veterinary inspection records at slaughterhouses, and some cases may be unnoticed, especially in mild infections. Thus, the use of serological tests with sensitivity higher than the routine postmortem inspection has been recommended as an option for antemortem detection of bovine cysticercosis, allowing a more accurate early identification of infected animals (PAULAN et al., 2013; GUIMARÃES-PEIXOTO et al., 2015). Positive findings for bovine cysticercosis based on absolute numbers of occurrences may lead to misinterpretation of the disease spatial distribution, since regions with high concentrations of these events are not always the areas with the highest risk (BAVIA et al., 2012). Therefore, epidemiological maps of disease risk have been produced in order to correlate disease data with environmental features at sites known to harbor bovine cysticercosis. However, studies on the distribution of bovine cysticercosis in Brazil have only taken postmortem inspections into account and not serological tests (BAVIA et al., 2012; DUTRA et al 2012; ROSSI et al., 2016). Thus, so far, no surveys involving herd-level spatial clustering analysis on bovine cysticercosis seroprevalence in Brazil have been conducted.

Spatial clustering analysis is a useful tool for studying the spread of infectious diseases in animal populations. The identification of clusters might yield important information about the transmission and/or control measures for such diseases (CARPENTER, 2001). Thus, in the present study, a spatial cluster analysis was performed with the aim of determining the spatial distribution of bovine cysticercosis in the state of Paraíba, northeastern Brazil.

## Materials and Methods

### Sampling

The data used in the present study originated from an epidemiological survey for bovine cysticercosis in the state of Paraíba (MAIA, 2016). The state of Paraíba was divided into three sampling groups: sampling stratum 1 (Sertão mesoregion), sampling stratum 2 (Borborema mesoregion), and sampling stratum 3 (Zona da Mata and Agreste mesoregions) (Figure 1). For each sampling stratum, a pre-established number of herds were randomly selected (primary sampling units) and then, a pre-established number of cows aged  $\geq 24$  months were randomly selected (secondary sampling units).

The number of herds selected per sampling stratum was determined by simple random samples (THRUSFIELD, 2007) adopting 95% confidence level, 1.1% estimated herd-level prevalence (SANTOS et al., 2013) and 5% error. Nevertheless the veterinarians and agricultural and livestock technicians of the Secretariat of Agricultural and Fisheries Development of the State of Paraíba (SEDAP) were involved in the fieldwork, so the operational and financial capacity of the SEDAP was taken into consideration and herd sample size was adjusted to their field capacity. For the secondary units, the minimum number of animals to be examined within each herd in order to allow it to be classified as positive was estimated using the concept of aggregate sensitivity and specificity (DOHOO et al., 2003), considering 81.25% (SILVA et al., 2015a) and 98.3% (SILVA et al., 2015b) for the sensitivity and specificity, respectively, for indirect ELISA and immunoblot tests serially applied and 31% for the intra-herd estimated prevalence (ASAAVA et al., 2009). The Herdacc version 3 software (JORDAN, 1995) was used during this process, and the sample size was selected so that the herd sensitivity and specificity values would be  $\geq 90\%$ . Therefore, 10 animals were sampled in herds with up to 99 cows aging over 24 months; 15 animals were sampled in herds with 100 or more cows aging over 24 months; and all animals were sampled in those with up to 10 cows aging over 24 months. Cows within the herds were selected systematically. In total, 2382 animals in 474 cattle herds were sampled.

The target condition was a seropositive animal within an infected herd. The herd-level case definition was based on the size of the population (cows aging  $\geq 24$  months), number of females sampled, an intra-herd apparent prevalence of 31% (ASAAVA et al., 2009), and the sensitivity and specificity of the diagnostic tests that were used serially (indirect ELISA and immunoblot), with the goal of achieving herd sensitivity and specificity of  $\geq 90\%$ . After new simulations using the Herdacc software, a herd was deemed positive for cysticercosis if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females.

### Serological diagnosis

The serological diagnosis of bovine cysticercosis was initially done by using indirect ELISA and positive serum samples were confirmed by the immunoblot test. Both tests were carried out in accordance with methodologies previously described by Pinto et al. (2000) and Silva et al. (2015a, b), using *T. crassiceps* larvae as antigens. For indirect ELISA, the positivity and negativity of each sample was determined by calculating the cut-off points which were defined as the mean of the optical densities (OD) of the reactions of the negative control serum samples, plus two standard deviations.

### Statistical analysis

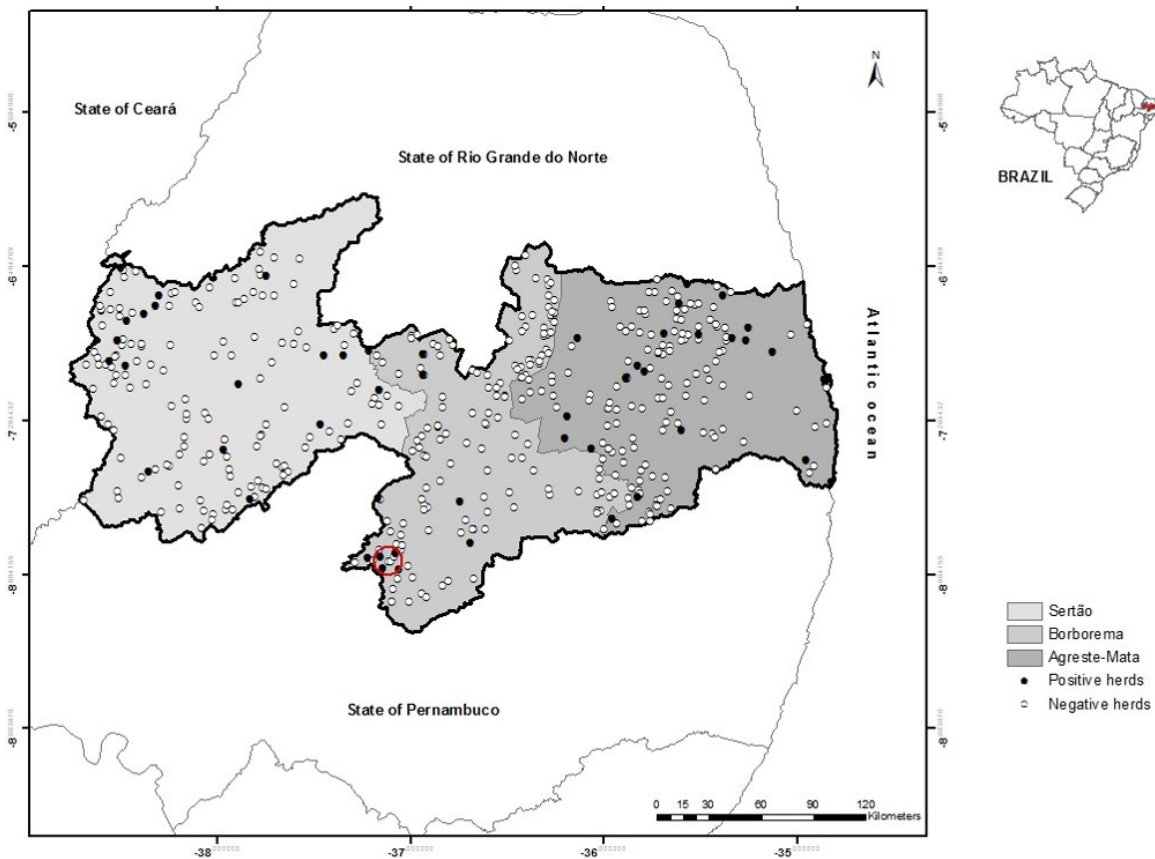
The spatial clustering of bovine cysticercosis-positive herds was assessed using two methods (WARD & CARPENTER, 2000). First, the Cuzick-Edwards  $k$ -nearest neighbor method (CUZICK & EDWARDS, 1990) was used to detect the possibility of global spatial clustering at herd level using the ClusterSeer 2.5.1 software

(BioMedware, Ann Arbor, MI, United States). The existence of potential spatial clustering was analyzed at each of the first 10 neighborhood levels, and the overall p-value was adjusted for multiple comparisons using the Simes approach. Furthermore, scan statistics generated through the SatScan software version 9.0 (KULLDORFF & NAGARWALLA, 1995) were used to identify local clusters of positive herds. A Bernoulli model was applied, the scanning window was circular and the spatial size of the scan window was limited to 25% of the total population.

## Results and Discussion

The herd-level prevalence in the state of Paraíba was 10.8% (95% CI = 8.1%-14.1%), 10.3% (95% CI = 6.4%-16.1%) in the Sertão region, 6.9% (95% CI = 3.9%-12.1%) in the Borborema

region and 13.8% (95% CI = 9.3%-20.2%) in the Agreste and Zona da Mata regions (Table 1). The herd-level prevalence found in the State of Paraíba, especially in the Agreste/Zona da Mata and Sertão mesoregions, where herd-level prevalences were 13.8% (95% CI = 9.3%-20.2%) and 10.3% (95% CI = 6.4%-16.1%), respectively, indicate that bovine cysticercosis is spread in cattle herds in the region. Data on bovine cysticercosis prevalence using serological methods as diagnostic tests in Brazil are scarce and limited to local surveys. Seropositivities rates at animal level have been referred to range from 0.4% to 4.1% in surveys conducted in the state of Minas Gerais (IASBIK et al., 2010; ACEVEDO et al., 2012; SANTOS et al., 2013; FELIPPE et al., 2014; GARRO et al., 2015). It seems that the herd-level prevalence could be even higher in Paraíba, once for this study only cows aged  $\geq 24$  months were



**Figure 1.** Significant cluster (red line) of bovine cysticercosis positive herds in the State of Paraíba. Detail shows Paraíba State within Brazil.

**Table 1.** Census data on the cattle population according to sampling stratum and herd-level prevalence of bovine cysticercosis, in the state of Paraíba, northeastern Brazil.

Sampling stratum	Total no. of herds	No. of herds		Prevalence (%)	95% CI
		Tested	Positive		
Sertão	24,356	156	16	10.3	[6.4-16.1]
Borborema	11,603	159	11	6.9	[3.9-12.1]
Agreste/Zona da Mata	18,398	159	22	13.8	[9.3-20.2]
State of Paraíba	54,357	474	49	10.8	[8.1-14.1]

used. Nevertheless, within-herd prevalence ranged from 7.1% to 100% (median of 16.7%).

No significant clusters were identified (Simes  $p > 0.05$ ) through the Cuzick and Edwards method for the entire state of Paraíba. However, when the state was divided into separate strata, significant global clustering (Simes  $p < 0.05$ ) of positive herds was detected through the Cuzick and Edwards method at the  $k = 3$  neighborhood level in the Borborema mesoregion. Using the Bernoulli model, a spatial cluster of positive herds was detected in the southern part of the Borborema mesoregion (Figure 1). In this cluster, the number of herds was 7, the radius of the cluster was 8.02 km and the numbers of observed and expected cases (positive herds) were 5 and 0.53, respectively, such that the risk of infection was 15.4 (relative risk = 15.4;  $p = 0.008$ ) times higher in herds located inside the cluster than in those located elsewhere. Allepuz et al. (2009) identified two statistically significant clusters of bovine cysticercosis in the Catalonia region (northeastern Spain) and concluded that the location of the farm may also have an influence on the risk of bovine cysticercosis. These authors suggested that the large number of infected animals and the fact that the animals originated from different regions in Spain and different countries in Europe, practically ruled out the possibility of that the animals had become infected at their origin.

In the present study, there was a lack of spatial clustering of bovine cysticercosis throughout the state of Paraíba, but a spatial cluster was identified considering each mesoregion independently. However, it can be inferred that this cluster cannot be explained by spatially structured factors as reported by Ávila et al. (2013), who detected clustering for bovine tuberculosis in the state of Bahia only when the regions were analyzed separately. The geographical divisions (Sertão, Borborema and Agreste/Zona da Mata) that were created in the present study for analysis purposes were not subject to the real parameters regarding occurrences of cysticercosis and did not respect geographical boundaries. Thus, the cluster found in the Borborema region can be explained by its location at the border with the State of Pernambuco. More precisely, it is close to the place where there is the second largest cattle fair in the state, in the municipality of Tabira. Large-scale movements of animals from different locations occur at this fair, which may result in greater numbers of traded animals that may have cysticercosis.

In the state of Paraíba, most farms are family-run or subsistence farms, with predominantly mixed production and semi-intensive farming (CLEMENTINO et al., 2015). This leads to inappropriate practices such as self-consumption of the farm's meat or selling it within the community, without any sanitary inspection (ARAGÃO et al., 2010). Thus, the cattle may be exposed to important environmental risk factors for bovine cysticercosis, such as surface water, flooded pastures and grazing on pastures contaminated with *T. saginata* eggs from human feces, which favors persistence of the taeniasis-cysticercosis complex (BARBOSA et al., 2001; BOONE et al., 2007).

Detection of spatial clustering involves complex methodology and has limitations but it can bring more accurate results and help decision-making, for greater efficiency of sanitary measures as pointed out by Ávila et al. (2013). In the present context, it is not plausible to suggest testing cows prior to purchasing them because serological tests for bovine cysticercosis are costly,

not widely available, and do not differentiate between previous and active infection. Moreover, replacement or maintenance of livestock by animal purchases is common in the region. Therefore, measures should be based on disease prevention at herd level, such as avoidance of contact with human feces and contaminated water and food, among cattle (MURRELL et al., 2005).

Taking into account the multiplicity of factors that is involved in the transmission of bovine cysticercosis (BAVIA et al., 2012), and the high prevalence of bovine cysticercosis in the state of Paraíba further epidemiological surveys should be conducted among both humans and cattle, with the aim of identifying possible conditions that could act as risk factors for the occurrence and distribution of bovine cysticercosis in this region.

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